Pharmacology, Biochemistry and Behavior 100 (2012) 413–418

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Asiaticoside: Attenuation of neurotoxicity induced by MPTP in a rat model of Parkinsonism via maintaining redox balance and up-regulating the ratio of Bcl-2/Bax

Chang-Liang Xu [a], Qi-Zhi Wang [b], Ling-Mei Sun [c], Xiu-Min Li [a], Ji-Min Deng [a], Lu-Fan Li [a], Jin Zhang [a], Rong Xu [d], Shi-Ping Ma [a,*]

[a] Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing 210009, PR China
[b] Jiangsu Institute of Botany, Chinese Academy of Sciences, PR China
[c] Department of Biology, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA
[d] Department of Pharmacology of Traditional Chinese Medical Formulae, Nanjing University of Traditional Chinese Medicine, Nanjing 210029, PR China

ARTICLE INFO

Article history:
Received 31 May 2011
Received in revised form 15 September 2011
Accepted 27 September 2011
Available online 6 October 2011

Keywords:
Asiaticoside
Parkinson's disease
Neuroprotection
Bcl-2/Bax ratio
Redox

ABSTRACT

In this study, we investigated the neuroprotective effects of asiaticoside, a triterpenoid saponin isolated from the Chinese medicinal herb *Centella asiatica*, in the rats model of Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Rats were first injected with MPTP. One day after surgery, asiaticoside was administered and the behavioral tests were assessed. On 14th day, the rats were sacrificed, substantia nigra (SN) and striatum were dissected, and then dopamine (DA) and its metabolites in striatum and malonyldialdehyde (MDA) contents, reduced glutathione (GSH) level and gene expression level in SN were estimated. Treatment with asiaticoside was found to protect dopaminergic neuron by antagonizing MPTP induced neurotoxicity and to improve locomotor dysfunction. Asiaticoside significantly attenuated the MPTP-induced reduction of dopamine in the striatum. The content of MDA was significantly decreased while the GSH level was significantly increased in asiaticoside-treated groups. In addition, asiaticoside increased the Bcl-2/Bax ratio. These results indicated that asiaticoside was effective in reversing MPTP induced Parkinsonism via its neuroprotective effects including antioxidant activity, maintaining the metabolic balance of DA, and increasing ratio of Bcl-2/Bax.

1. Introduction

Parkinson's disease (PD) is a chronically progressive, age-related neurodegenerative movement dysfunction characterized by progressive resting tremor, rigidity, bradykinesia, postural instability along with non-motoric symptoms like autonomic, cognitive and psychiatric problems (Mandemakers et al., 2007). The neuropathological hallmarks are characterized by massive loss of nigrostriatal dopaminergic neurons in the substantia nigra (SN) pars compacta and the resultant deficiency in the neurotransmitter dopamine (DA) at the nerve terminals in the striatum (Nagatsu and Sawada, 2005). Several hypotheses have been proposed for explaining the progressive and selective neurodegeneration in PD. Mitochondrial dysfunction, which is one of most important hypotheses, has been linked with PD for a long time. The dysfunction of mitochondria, including deficiencies in energy supply, free radical generation, Ca^{2+} buffering, not only involve damage to the organelle and loss of bioenergetic function but also disruption of mitochondrial-dependent redox signaling pathways, and eventually lead to cell death (Gutierrez et al., 2006).

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) elicited Parkinsonism was first observed in a group of heroin users presented with symptoms similar to PD (Langston et al., 1983). Following research found that the biochemical and cellular changes that occur after administration of MPTP in animals were remarkably similar to those seen in idiopathic PD (Ferro et al., 2005). DA supplementation therapy by L-dopa for PD was established around 1970. However, many patients develop motor complications, and L-dopa-induced dyskinesia is common and difficult to treat. So a much more effective compound that have less side effects is needed to be found out in order to take place of traditional DA supplementation therapy by L-dopa.

Asiaticoside (Fig. 1), a triterpenoid saponin, is isolated from *Centella asiatica* which has a long history of use in India as a memory enhancing drug in ayurvedic literature and has been proved to have some pharmacological activities in central nervous system (Dhanasekaran et al., 2009; Flora and Gupta, 2007; Haleagrahara and Ponnusamy, 2010). Asiaticoside also offers protection against chemical-induced inflammation and hepatotoxicity (Yun et al., 2008; Zhang et al., 2010). Moreover, recent studies indicated that

Abbreviations: PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SN, substantia nigra; DA, dopamine; MDA, malonyldialdehyde; GSH, reduced glutathione; qRT-PCR, quantitative real-time polymerase chain reaction; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid; DHBA, 3, 4-dihydroxybenzylamine; HPLC, high-performance liquid chromatography; LPO, lipid peroxidation; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

* Corresponding author. Tel.: +86 25 83271419; fax: +86 25 83271505.
E-mail address: mashiping1956@yahoo.cn (S.-P. Ma).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.09.014

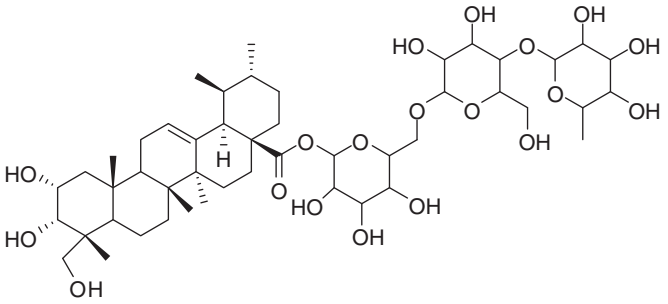

Fig. 1. The structure of asiaticoside.

asiaticoside has shown to rescue B103 rat neuroblastoma cells against $A\beta_{25-35}$ and H_2O_2-induced neurotoxicity (Mook-Jung et al., 1999). MPTP rats have been used as a model of Parkinsonism for evaluating the anti-PD effect of asiaticoside. The effects of asiaticoside on locomotor activity, DA content, malondialdehyde (MDA) concentrations, and reduced glutathione (GSH) levels in nigrostriatal system of MPTP-induced Parkinsonism phenotype in rats were investigated. Additionally, the expressions of Bcl-2 and Bax were also evaluated for elucidating the underlying molecular mechanisms of neuroprotection of asiaticoside.

2. Materials and methods

2.1. Apparatus and reagents

The high-performance liquid chromatography (HPLC) system with electrochemical detection consisted of a solvent delivery module, an ESA sampler, an ODS reverse-phase column (C18 250 mm × 4.6 mm) and an ESA-Coulochem III electrochemical detector with a 5020 guard cell and a 5010 analytical cell. DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and internal standard 3, 4-dihydroxybenzylamine (DHBA) were purchased from Sigma (USA). All solutions were prepared with ultra pure water (18.2 MΩ-cm) from a Purelab ultra System (ELGA Purelab, UK).

Asiaticoside was kindly provided by Dr. Qizhi Wang (Jiangsu Institute of Botany, Chinese Academy of Science) and the purity of this compound was greater than 97% tested by HPLC analysis.

2.2. Animals

Adult male Wistar rats (170–190 g, Shanghai laboratory Animal Center, Chinese Academy of Science) were used in this study. The animals were fed in a controlled environment (23 ± 1 °C, 12-h light–dark cycle light) with *ad libitum* access to food and water. The animals were cared complied with the Provisions and General Recommendation of the Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Jiangsu Province.

2.3. Experimental design

Rats were distributed randomly into 6 groups with 6 in each: control group, sham-operated control group, the MPTP (MPTP-lesioned group) group, asiaticoside treated groups (15, 30, or 45 mg/kg/day). All animals underwent stereotaxic surgery and bilateral infusion of MPTP or saline on day 0 into the SN. One day after surgery, the rats received daily intragastric administration (i.a.) of asiaticoside or saline in a volume of 1 ml/kg at 13:00 h for 14 days. Open-field test was conducted on the 1st, 7th and 14th days at 14:00–18:00 h. Ladder-walking was conducted on the 7th and 14th days at 14:00–18:00 h. At the end of these experiments, the rats were sacrificed and dissection of the striatum and SN for other experiments.

2.4. Stereotaxic surgery

The method of brain surgery has been reported previously (Ferro et al., 2005). In brief, the rats were anesthetized by intraperitoneal (i.p.) injection of chloral hydrate at a dose of 400 mg/kg, and then stereotaxically injected bilaterally into the SN with MPTP (Sigma, USA; 1 μmol in 2 μl of saline, 0.35 μl/min) through a 30-gauge needle according to the following coordinates: AP: −5.0 mm, ML: ± 2.1 mm, DV: −7.7 mm from the bregma, midline, and skull surface, respectively. Controls were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP. Immediately after surgery, the rats were injected with penicillin-G procaine (0.2 ml, 20,000 IU, IM) and the rats were left in a temperature-controlled chamber until they recovered from anesthesia, then they were returned to their home cages.

2.5. Behavioral test

2.5.1. Open-field test

Open-field consisted of square arena (120 × 120 cm). The square arena was divided into 16 sub squares. The rats were submitted to the open-field on the 1st, 7th and 14th days after the surgery. The test placed the rat in the center of the arena. The behaviors of the rats were then observed for 5 min. After each test, the apparatuses were cleaned. The number of crossings (The rats cross the sub squares boundaries with paws), peripheral ambulation time (The rats crawl time with their paws), and immobilization time were scored by an observer blind to the treatment received by each rat.

2.5.2. Ladder walking

Crossing the 'horizontal ladder' required the rats accurately place their limbs on the bars. The test was estimated coordinating ability of rats' hindlimb and forelimb. Rats were trained to walk across the ladder rung and the rungs were irregularly spaced out (1–3 cm apart). The rung space was not altered during all experimental days. Rats were trained to walk across the ladder for 3 days before the test and the average performance of three times in days 7 and 14 was recorded.

2.6. HPLC analysis DA and its metabolites

On the 14th day, the rats were decapitated and the brains were quickly removed within 30 s and immediately dropped into ice-cold saline. The brains were dissected over ice. Each group we randomly choose three rats whose striatum from the left hemisphere were weighted and homogenized at perchloric acid (0.1 M) in a glass homogenizer. The levels of DA and its metabolites were detected by HPLC analysis previously described by our laboratory (Li et al., 2011). The results were calculated and expressed as ng/mg tissue weight.

2.7. Assay of substantia nigra MDA level

The lipid peroxidation (LPO) of SN was studied by measuring the malonyldialdehyde (MDA) level by a colorimetric method involving thiobarbituric acid (TBA) adduct formation. Homogenate of SN was prepared, and the amounts of TBA reactive substances (TBARS) such as MDA were measured by the reaction with TBA using a commercial TBARS Assay Kit (Cayman Chemical Co.). Operation followed the manufacturer's protocol. The MDA were determined by comparison with standards and normalized to protein content.